A 1 bp Deletion in the Dual Reading Frame Deafness Gene LRTOMT Causes a Frameshift From the First Into the Second Reading…

Article *in* American Journal of Medical Genetics Part A · August 2011

DOI: 10.1002/ajmg.a.34096 · Source: PubMed

8 authors, including:

Isabelle Schrauwen
Baylor College of Medicine
55 PUBLICATIONS **623** CITATIONS

SEE PROFILE

Ellen P A Jorssen
University of Antwerp
19 PUBLICATIONS **153** CITATIONS

SEE PROFILE

Morteza Hashemzadeh Chaleshtori
Shahrekord University
62 PUBLICATIONS **499** CITATIONS

SEE PROFILE

RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

A 1 bp Deletion in the Dual Reading Frame Deafness Gene *LRTOMT* Causes a Frameshift From the First Into the Second Reading Frame

Maarten Vanwesemael,[1] Isabelle Schrauwen,[1] Ruben Ceuppens,[1] Fatemeh Alasti,[1] Ellen Jorssen,[1] Effat Farrokhi,[2] Morteza Hashemzadeh Chaleshtori,[2] and Guy Van Camp[1]*

[1]Department of Medical Genetics, University of Antwerp, 2610, Wilrijk, Belgium

[2]Cellular and Molecular Research Center, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

Received 29 November 2010; Accepted 12 April 2011

TO THE EDITOR:

Congenital hearing impairment (HI) affects one in 650 newborns and has a genetic cause in 50% of the cases. Autosomal recessive non-syndromic hearing impairment (ARNSHI) is responsible for 70–80% of all hereditary cases of HI. ARNSHI is genetically highly heterogeneous and until today mutations in 37 genes have been identified [Van Camp and Smith, 2011]. One of the latest genes for ARNSHI is *LRTOMT* (at locus *DFNB63* on 11q13.3-q13.4). The structure of human *LRTOMT* has only recently been elucidated [Ahmed et al., 2008]. *LRTOMT* is an evolutionary recent fusion gene with alternative reading frames that exclusively exists in primates and is a fusion of non-primate *Lrrc51* (Leucine rich repeat containing 51) and *Tomt* (Transmembrane *O*-methyltransferase) sequences. Human *LRTOMT* has ten exons, of which the first two are non-coding. Five transcripts are known and it has two different major protein products, LRTOMT1 and LRTOMT2. Both proteins contain a predicted transmembrane domain, located in exon 5 of LRTOMT1 and in exon 8 of LRTOMT2. *LRTOMT2* is also described as *COMT2* (Catechol-*O*-methyltransferase) [Du et al., 2008].

In this study we analyzed a five-generation consanguineous Iranian pedigree with ARNSHI (Fig. 1). All affected family members in the pedigree (aged 34, 45, 49, and 54) showed a congenital profound sensorineural hearing loss. History and physical examination of participating family members did not reveal environmental factors as a cause for the HI and confirmed the non-syndromic nature of the HI.

Family history and clinical information were collected through interviews and physical examination of family members. Pure tone air conductance audiometry was performed on all affected family members at frequencies between 250 and 8,000 Hz. Informed consent was obtained from all participating family members and parents of under-aged members. Venous blood was obtained of nine family members. Genomic DNA was extracted using a standard phenol chloroform protocol. Genotyping of microsatellite markers was performed by polymerase chain reaction (PCR). PCR-products were fluorescently labeled and subsequent fragment analysis was performed on an ABI 3130XL DNA sequence analyzer (Applied Biosystems Inc., Foster City, CA), using standard procedures.

How to Cite this Article:
Vanwesemael M, Schrauwen I, Ceuppens R, Alasti F, Jorssen E, Farrokhi E, Chaleshtori MH, Van Camp G. 2011. A 1 bp deletion in the dual reading frame deafness gene *LRTOMT* causes a frameshift from the first into the second reading frame.
Am J Med Genet Part A 155:2021–2023.

A subset of all known ARNSHI loci (DFNB1, 2, 3, 4, 6, 7/11, 8, 9, 12, 21, 22, 23, 28, 35, 59, 63) with three markers per locus was analyzed, and linkage to DFNB63 was found. This subset was based upon reports on the prevalence of mutations in Iranian and in other populations [Hilgert et al., 2009]. Homozygosity was only consistent at the DFNB63 locus on 11q13.3-q13.4 [Khan et al., 2007] and, the other loci were excluded. A multipoint LOD-score of 2.83 was calculated (SimWalk v2.91). All affected family members were homozygous for five extra markers (D11S4140, D11S4184, D11S4196, D11S1314, and D11S4132) at the DFNB63 locus (Fig. 1). The causative gene for this locus is *LRTOMT* [Ahmed et al., 2008].

The coding region (10 exons) and exon–intron boundaries of *LRTOMT* were sequenced on the basis of genomic DNA. *LRTOMT*

Grant sponsor: Flemish FWO; Grant number: G.0138.07; Grant sponsor: Cellular and Molecular Research Center, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran; Grant number: 534.
*Correspondence to:
Guy Van Camp, Department of Medical Genetics, University of Antwerp, Universiteitsplein 1 2610 Wilrijk, Belgium E-mail: guy.vancamp@ua.ac.be
Published online 7 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34096

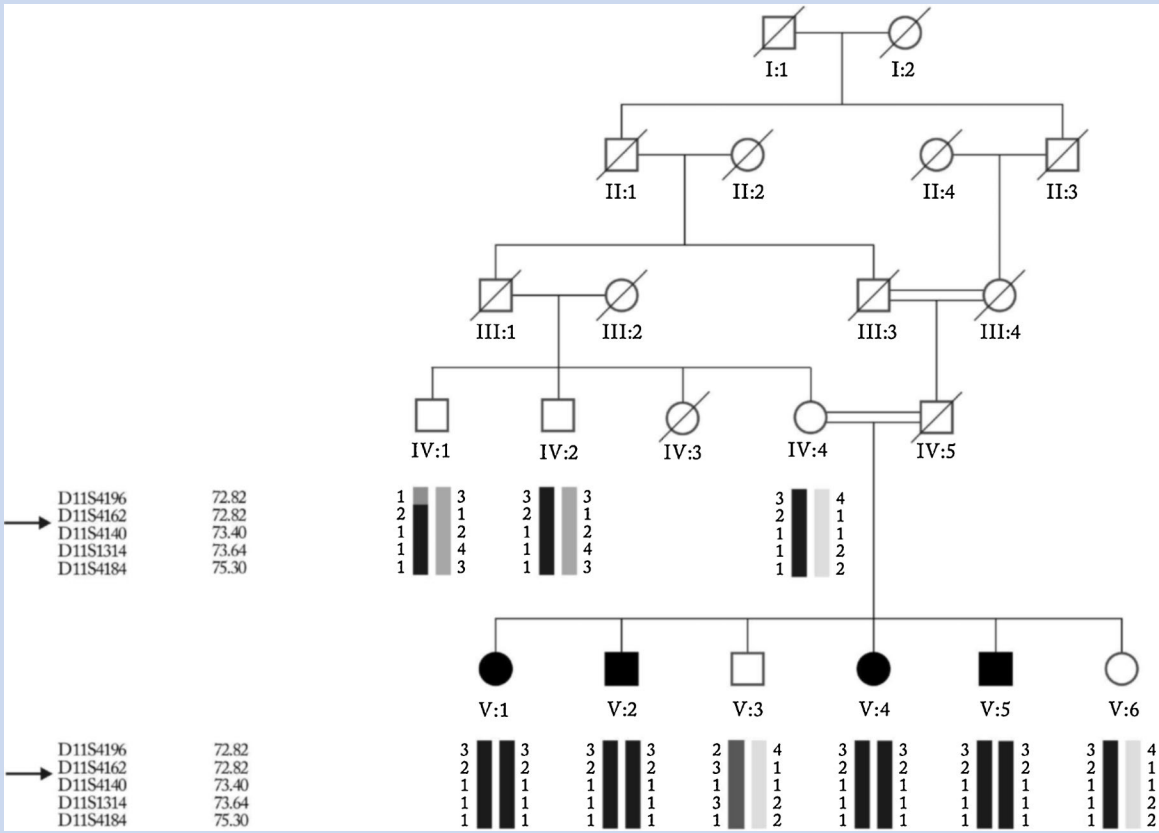

FIG. 1. Pedigree of the family. Haplotype analysis defines a homozygous region at the *DFNB63* locus. Genetic markers and chromosomal location (cM) according to the Marshfield map (http://research.marshfieldclinic.org/genetics) are indicated on the left. The black haplotype indicates the linked haplotype. The arrow indicates the position of *LRTOMT*.

primers were used as published [Ahmed et al., 2008], except for exon 10, for which primers were designed using Primer-BLAST (NCBI, 2009). A combination of three overlapping PCR products was used to sequence the coding region and the exon–intron boundaries of exon 10. Primers used were CTACGTGACCTTGAGCAAAGC (1F) and AGGTCAGAGGTGGGAAGAGAG (1R), CAAGCAGGGACCTCAAAATC (2F) and AGTACCTCACCCCTTGATTGC (2R), TAGACATGGGTGGAAAATCACTC (3F), and TAGGGCTTACAGGCTCTGTCC (3R) After PCR, the samples were purified and sequenced with Big Dye Terminator chemistry (Applied Biosystems Inc.) on an ABI 3130XL DNA sequence analyzer (Applied Biosystems Inc.). One affected family member and one unaffected family member were sequenced for all exons and exon–intron boundaries. Exon 8 was sequenced in all nine family members.

DNA sequencing of exons and exon–intron boundaries of this gene revealed a homozygous c.104delC mutation in all affected family members. Together with six earlier reported mutations [Ahmed et al., 2008; Du et al., 2008], there are now seven different mutations known, all associated with ARNSHI (Table I). Four reported missense mutations cause amino acid substitutions of evolutionary well-conserved residues in the catechol-*O*-methyltransferase domain of *LRTOMT2*/*COMT2*. One reported mutation, $358+4A>C$, results in skipping of exon 8 and for one mutation, L16P, the effect on protein expression and methyltransferase activity is less clear.

The c.104delC mutation we discovered causes a frameshift in *LRTOMT2* and leads to a new stop codon 12 amino acids downstream of the deletion, completely eliminating the catechol-*O*-methyltransferase domain and the major part of the transmembrane domain. Compromising the functionality of the catechol-*O*-methyltransferase domain has already been associated with ARNSHI, either through skipping of exon 8 or through the substitution of functionally important, conserved amino acids. The premature terminator codon makes the truncated *LRTOMT2* transcript a possible target for nonsense-mediated RNA decay.

Five out of the six previously reported mutations causing amino acid changes in LRTOMT2 cause no amino acid alteration in LRTOMT1; although three of them lie in the 3′UTR of *LRTOMT1*. The sixth mutation results in skipping of exon 8, which codes for both LRTOMT1 and LRTOMT2, and is likely to affect both proteins.

The c.104delC deletion is located at position 507 of the LRTOMT1 cDNA and thus also has consequences for the 3′-end of *LRTOMT1*, as the frameshift eliminates the normal stop codon in

TABLE I. List of Currently Known Mutations in *LRTOMT*

LRTOMT2 cDNA level[a]	*LRTOMT2* Protein level	*LRTOMT1* cDNA level[a]	*LRTOMT1* Protein level	Population	Reference
c.47T>C	p.Leu16Pro	c.450T>C	p.Thr150Thr	Iranian	Du et al., 2008
c.333C>G	p.Tyr111X	c.736C>G	3′UTR	Iranian	Du et al., 2008
c.358+4A>C	p.Ala29SerfsX54	c.761+4A>C	p.Gly163ValfsX4	Turkish	Ahmed et al., 2008
c.242G>A	p.Arg81Gln	c.645G>A	p.Ala215Ala	Tunisian	Ahmed et al., 2008
c.313T>C	p.Trp105Arg	c.716T>C	3′UTR	Tunisian	Ahmed et al., 2008
c.328G>A	p.Glu110Lys	c.731G>A	3′UTR	Pakistani	Ahmed et al., 2008
c.104delC	p.Ser35SerfsX13	c.507delC	p.Val169ValfsX258	Iranian	This study

[a]Nucleotides are numbered from the first coding ATG based on Ahmed *et al.*, 2008.

exon 8. Due to the deletion translation shifts from the *LRTOMT1* into the *LRTOMT2* reading frame resulting in a C-terminus derived from LRTOMT2.

The deletion is thus predicted to result in a chimeric protein product that contains a part of *LRTOMT1* (N-terminus) and a part of the *LRTOMT2* (C-terminus). This chimeric protein includes the LRTOMT2 catechol-*O*-methyltransferase domain.

In summary, we describe a unique frameshift mutation in a dual reading frame gene *LRTOMT*. This results in elimination of the catalytic domain of the second transcript (LRTOMT2), which is most likely disease causing. Additionally, the first transcript (LRTOMT1) now contains part of both *LRTOMT1* (5′) and *LRTOMT2* (3′) reading frames. It is not clear whether this transcript is translated into the predicted chimeric protein.

ACKNOWLEDGMENTS

We would like to thank the family members for their participation and cooperation. This work was supported by the Flemish FWO (grant G.0138.07) and the Cellular and Molecular Research Center, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran (grant number 534). IS is a postdoctoral fellow of the FWO.

REFERENCES

Ahmed ZM, Masmoudi S, Kalay E, Belyantseva IA, Mosrati MA, Collin RW, Riazuddin S, Hmani-Aifa M, Venselaar H, Kawar MN, Tlili A, van der Zwaag B, Khan SY, Ayadi L, Riazuddin SA, Morell RJ, Griffith AJ, Charfedine I, Caylan R, Oostrik J, Karaguzel A, Ghorbel A, Riazuddin S, Friedman TB, Ayadi H, Kremer H. 2008. Mutations of LRTOMT, a fusion gene with alternative reading frames, cause nonsyndromic deafness in humans. Nat Genet 40:1335–1340.

Du X, Schwander M, Moresco EM, Viviani P, Haller C, Hildebrand MS, Pak K, Tarantino L, Roberts A, Richardson H, Koob G, Najmabadi H, Ryan AF, Smith RJ, Muller U, Beutler B. 2008. A catechol-*O*-methyltransferase that is essential for auditory function in mice and humans. Proc Natl Acad Sci USA 105:14609–14614.

Hilgert N, Smith RJ, Van Camp G. 2009. Forty-six genes causing non-syndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutat Res 681:189–196.

Khan SY, Riazuddin S, Tariq M, Anwar S, Shabbir MI, Riazuddin SA, Khan SN, Husnain T, Ahmed ZM, Friedman TB. 2007. Autosomal recessive nonsyndromic deafness locus DFNB63 at chromosome 11q13.2-q13.3. Hum Genet 120:789–793.

NCBI. 2009. Primer-BLAST. http://www.ncbi.nlm.nih.gov/tools/primer-blast/.

Van Camp G, Smith RJ. 2011. Hereditary Hearing loss Homepage. http://hereditaryhearingloss.org/.